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TRANSFORMATION OF *BACILLUS LICHENIFORMIS*[1]

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ABSTRACT

GWINN, DARREL D. (Oregon State University, Corvallis), AND CURTIS B. THORNE. Transformation of *Bacillus licheniformis*. J. Bacteriol. **87**:519–526. 1964.—When a series of 28 auxotrophic mutants of *Bacillus licheniformis* were screened for transformation, only three of them, M28 (glycine$^-$), M30 (uncharacterized), and M33 (purine$^-$), produced a detectable number of transformants. The screening method consisted of spreading auxotrophic cells and deoxyribonucleic acid (DNA) from the prototrophic strain 9945A on minimal agar plates and observing the plates for development of prototrophic colonies. M28 transformed at a higher frequency than did the two other mutants, and it was studied in greater detail. Although up to 20% of the recipient cells spread on the plates in the presence of DNA gave rise to prototrophic colonies over a period of 72 hr, only about 10^{-3}% of the cells produced transformants when they were incubated with DNA in liquid suspension for 1 hr. The most competent cultures of many tested were those grown on a shaker for 22 hr in a medium composed of nutrient broth, salts, and glycerol. When mutations resulting in requirements for histidine, leucine, serine, and trytophan were introduced singly into the glycine mutant, transformants for the leucine, serine, and histidine markers could be obtained at will, but transformants for the tryptophan marker were not detected even though all four of the double mutants could be transformed to glycine independence.

Bacillus licheniformis strain 9945A synthesizes a large amount of glutamyl polypeptide during growth in a synthetic medium (Thorne et al., 1954). We became interested in transformation of this organism when we began a study of genetic factors affecting glutamyl polypeptide synthesis. Two transducing phages for *B. licheniformis* were available (Taylor and Thorne, 1963) but, in view of the low frequencies of transduction which were characteristic of this system and without a specific method for selecting cells that were transduced for the ability to synthesize peptide, we turned to transformation with the hope that higher frequencies of genetic transfer could be obtained. Although transformation has been reported for *B. subtilis* (Spizizen, 1958), *B. natto*, and *B. subtilis* var. *aterrimus* (Marmur, Seaman, and Levine, 1963), we are not aware of any previous report on transformation of *B. licheniformis*. We spent a great deal of effort in obtaining a transforming system with this organism, and the following is an account of some of our efforts, in which we were able to transform only a few auxotrophic mutants from a series of many that were tested.

MATERIALS AND METHODS

Bacterial strains. *B. licheniformis* ATCC 9945A and 10716 were used. *B. subtilis* strains W-23-S^r (a streptomycin-resistant mutant of wild-type W-23) and 168 (indole$^-$) were obtained from Maurice Fox. Auxotrophic mutants were isolated by the method of Iyer (1960) after irradiation of spores with ultraviolet light.

Media and cultural conditions. NBY medium was composed of 8 g of nutrient broth (Difco) and 3 g of Difco yeast extract per liter. The minimal medium was that of Thorne (1962), and it was always supplemented with 1 g of L-glutamic acid per liter. NBSG medium was prepared by adding 8 g of Difco nutrient broth and 5 g of glycerol (sterilized separately) per liter of minimal medium with glucose omitted. When solid media were desired, 15 to 25 g of agar per liter were added.

Except when stated otherwise, cells for transformation were grown from spore inoculum (about 10^8 spores) in 50 ml of medium contained in 250-ml Erlenmeyer flasks on a rotary shaker (230 rev/min describing a circle 1-in. in diameter). Viable counts were made by plating on NBY agar.

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Peptone (Difco; 0.5%, w/v) was the standard diluent for cells. Incubations were at 37 C unless stated otherwise.

Spores were grown on agar slants of potato medium (Thorne, 1962) or in PA broth [medium used by Thorne (1962) for assaying phage but without agar]. Cultures were incubated for 3 to 5 days until most of the cells had sporulated. Spores were collected by centrifugation, washed twice with water, and finally suspended in water and heated at 65 C for 30 min. They were stored in screw-cap tubes at 4 C.

Deoxyribonucleic acid (DNA). DNA was isolated by a procedure adapted from that described by Marmur (1961). Each of two 500-ml Erlenmeyer flasks containing 100 ml of broth was inoculated with 10^8 spores of the donor strain, and the flasks were incubated on a shaker at 37 C for 16 hr. The resulting cultures were pooled and centrifuged at 4,080 × *g* for 15 min. The cells were suspended in 5 ml of saline-EDTA (0.15 M NaCl plus 0.1 M ethylenediaminetetraacetate, pH 8) in a screw-cap tube, and 1 ml of lysozyme (40 mg/ml; 3× crystallized preparation from egg white obtained from Calbiochem) was added. The mixture was incubated with occasional shaking in a 37-C water bath for 45 min. A 0.4-ml portion of a saturated solution of sodium lauryl sulfate in 45% ethanol was added, and incubation was continued for 10 min with occasional mixing. Treatment with perchlorate, shaking with chloroform-isoamyl alcohol, centrifugation of the emulsion, and precipitation once with ethanol were carried out as described by Marmur (1961). The precipitate was dissolved in 2 M NaCl and was not purified any further.

Most of the DNA preparations were from cells grown in NBY broth, and such preparations always contained viable spores. They were sterilized by adding melted phenol (30%, v/v, final concentration) to the DNA in 2 M NaCl and incubating with occasional shaking in a 65-C water bath for 30 min. The DNA was then collected after a final precipitation from 95% ethanol and dissolved in 2 M NaCl for storage at 4 C. Later, we learned that cells sporulated very poorly in NBSG, and DNA prepared from such cells (16-hr cultures) was usually free of spores and thus did not require the phenol treatment. Results of tests with untreated and phenol-treated samples from the same preparation of DNA indicated that the phenol treatment did not affect transforming activity. The method of Burton (1956) was used to determine DNA.

Screening auxotrophs for transforming ability. The screening method consisted of spreading 0.1 ml of a 16-hr culture grown in NBY or NBSG and 0.05 ml (45 μg) of DNA, prepared from wild-type 9945A, on a minimal agar plate. Control plates included one with cells alone and one with cells and DNA plus 0.05 ml (100 μg) of deoxyribonuclease (1 × crystallized product of Worthington Biochemical Corp., Freehold, N.J.). The plates were incubated at 37 C and observed daily for 4 or 5 days. This process is sometimes referred to as "transformation on plates" or the "plate method of transformation."

Transformation of cells in liquid suspension. Routinely, experiments on transformation of cells in liquid suspension were set up in a manner similar to the following procedure. Recipient cells (usually 0.5 ml of a broth culture either undiluted or diluted in minimal broth) and 0.1 or 0.2 ml of DNA (10 to 50 μg) were incubated together in 6-in. test tubes in a 37-C water bath for 30 min. To show that DNA was the active principle, it was incubated with deoxyribonuclease (100 μg) for 15 min before cells were added. Incubating the DNA itself for 15 min did not affect its activity. As a control to test for spontaneous revertants, cells were incubated without DNA. Minimal broth was added when necessary to give a final volume of 0.7 ml in each of the tubes. Samples, usually 0.1 ml, were spread onto minimal agar plates for scoring transformants. To prevent transformation from occurring on the plates, 0.05 ml (100 μg) of deoxyribonuclease was spread with the DNA-cell mixtures.

When transformation mixtures were to be shaken, they were prepared as above, except that all volumes were doubled, and incubated in 30-ml serum bottles on a rotary shaker (described above).

RESULTS

Unsuccessful attempts to transform B. licheniformis auxotrophs. Most of the early tests were done with DNA isolated from wild-type 9945A and with cells of 9945A-M1 (thiamine$^-$), -M2 (lysine$^-$), -M3 (lysine$^-$), -M5 (serine$^-$), or -M8 (methionine$^-$) as recipients, since it was known that each of these could be transduced to prototrophy (Taylor and Thorne, 1963). Ini-

tially, the mutants were tested for transformation by following the procedure developed by Anagnostopoulos and Spizizen (1961) for transforming *B. subtilis*. Their method of growing recipient cells on Tryptose Blood Agar Base (Difco) followed by subsequent incubations of the cells in the minimal medium described by those authors was followed exactly. This procedure yielded no transformants with the five mutants listed above, although, in parallel tests with cells of *B. subtilis* 168 (indole$^-$) as recipients and DNA isolated from *B. subtilis* W-23-S^r, transformants were always obtained. An unsuccessful modification of the above method included the initial growth of recipient cells for 16 hr in shaken flasks of NBY broth rather than on Tryptose Blood Agar Base. Such cells were collected by centrifugation and resuspended in the minimal medium of those authors to give about 10^9 cells per ml.

Using the routine procedure described in Materials and Methods for transformation of cells in liquid suspension, we made other unsuccessful attempts to transform the five mutants of *B. licheniformis* listed above. Various ways of preparing recipient cells were tested including (i) growth from spore inocula as well as from vegetative inocula in NBY broth and NBSG broth in shaken flasks at 37 C for various periods of time ranging from 5 to 16 hr, (ii) growth for various periods of time at 37 C in shaken flasks of minimal medium supplemented with the appropriate growth factor, and (iii) growth in shaken flasks of NBY broth for 16 hr at 30 and 34 C. In other tests, cells grown for 16 hr in NBY broth were washed once with minimal broth and tested for transformation when resuspended in minimal broth adjusted to pH values ranging from 5 to 8. The procedure used by Stuy (1962) for transforming *Haemophilus influenzae* also failed to yield transformants with the above mutants of *B. licheniformis*.

Since these auxotrophs of strain 9945A did not transform under any of the conditions tested, we isolated auxotrophs from strain 10716 and tested them for transformation with DNA from wild-type 10716. Mutants requiring isoleucine and methionine, respectively, produced no transformants when they were tested by the procedure of Anagnostopoulos and Spizizen (1961).

Screening auxotrophs for transformation. We next proceeded to isolate additional auxotrophic mutants and to test them for transformation by the screening procedure given in Materials and Methods. The technique of transforming on agar plates had been found to produce transformants of *B. subtilis* 168 (indole$^-$) and of various auxotrophs of *B. subtilis* W-23-S^r. Fortunately, this approach turned out to be fruitful within a short period of time. When 28 auxotrophs were tested with wild-type DNA for transformation on plates, three of them, 9945A-M28 (glycine$^-$), -M30 (uncharacterized), and -M33 (purine$^-$), produced transformants. M28 transformed at a higher frequency than did M30 or M33, and for that reason it was studied in greater detail. The auxotrophs which did not give rise to transformants in this test included ones requiring thiamine, lysine, arginine, methionine, tryptophan, histidine, uracil, adenine, or hypoxanthine, and 13 with uncharacterized requirements.

Transformation of M28 confirmed. To confirm that the results obtained with M28 in the screening test were produced by transformation, experiments such as those shown in Table 1 were done. Transformants were produced with DNA isolated from wild-type 9945A but not with DNA isolated from M28. Spontaneous reversion to prototrophy was not detected in these experiments, and the addition of deoxyribonuclease to the DNA-cell mixtures prevented the occurrence of prototrophs. More transformants were produced from recipient cells grown in NBSG than from those grown in NBY. This might be related to the fact that fewer spores were produced in NBSG than in NBY. In a 16-hr culture of M28 grown in NBY, as many as 10% of the cells had sporulated whereas in a similar culture in NBSG fewer than 0.01% of the cells sporulated.

Colonial morphology. Colonies of M28 grew very slowly on NBY as well as on minimal agar supplemented with glycine. On NBY, colonies were circular with entire margins, and only about 2 mm in diameter. Colonies of wild-type 9945A on NBY agar were circular with erose margins, and much larger with a diameter of about 6 mm. On minimal medium supplemented with glycine, colonies of M28 were about 2 mm in diameter after 72 hr and appeared rough. In contrast, after only about 16 hr, wild-type 9945A colonies were large on minimal medium and very smooth as a result of glutamyl polypeptide formation. Such colonies accumulated mounds of peptide

TABLE 1. *Transformation of Bacillus licheniformis 9945A-M28 (glycine⁻) on agar plates**

Expt no.	Cells per plate	DNA		Deoxyribonuclease (μg/plate)	Transformants per plate†
		Source	Amt (μg/plate)		
1	1.2×10^7	9945A	45	0	580
			45	100	0
2	1.5×10^8	9945A	45	0	790
			45	100	0
			23	0	830
			4	0	520
			0.4	0	124
3	1.1×10^8	9945A	45	0	117
			45	100	0
		M28-RT	20	0	271
			20	100	0
		M28-ST	23	0	187
			23	100	0
		M28	15	0	0
4	3.0×10^7	9945A	40	0	>1,000–<2,000
			40	100	0
	3.0×10^6		40	0	>1,000–<2,000
	3.0×10^5		40	0	>1,000–<2,000
	4.5×10^4		40	0	626
	4.5×10^3		40	0	352
	4.5×10^2		40	0	90

* Recipient cells were from 16-hr shaken cultures grown in NBY for experiments 1, 2, and 3 and NBSG for experiment 4. The diluent for cells in experiment 4 was minimal broth. M28-RT is a rough prototrophic transformant and M28-ST is a smooth prototrophic transformant of M28. Control plates without DNA produced no colonies.

† When DNA from 9945A or M28-ST was used, about 10% of the transformants were smooth; with DNA from M28-RT, all the transformants were rough.

which ran over the agar surface when plates were tilted. Upon prolonged incubation for 96 to 120 hr, occasionally colonies of M28 on minimal medium supplemented with glycine exuded small amounts of viscous material which appeared to be glutamyl polypeptide. When medium E (Leonard, Housewright, and Thorne, 1958), a synthetic medium which supports production of large amounts of peptide by wild-type 9945A, was supplemented with glycine or yeast extract to allow growth of M28, the colonies were generally smooth, although they appeared not to produce as much peptide as the wild-type. Therefore, the term *rough* as applied to M28 refers to its colonial morphology on *minimal* medium in contrast to the very *smooth* appearance of the parent wild type.

Transformants of M28 produced with wild-type DNA were of two colonial types on minimal agar; about 10% of them were smooth, and the remainder were rough. When the recipient cells were grown in NBY, the first colonies appeared after about 48 hr of incubation, and these were the smooth transformants. The rough transformants, which grew more slowly, began to appear a few hours later. When recipient cells were grown in NBSG, the first colonies, which were smooth transformants, appeared in about 28 hr, and the final number of transformants was much greater than when the recipient cells were grown in NBY. Cultures from each of the two types of colonies were purified and designated M28-ST for the smooth transformant and M28-RT for the rough transformant. Colonies of M28-RT on minimal agar could not be distinguished from colonies of M28 on minimal

agar supplemented with glycine. Likewise, the cultures had similar colonial morphology on NBY agar. However, colonies of M28-ST on minimal agar or on NBY agar could not be distinguished from colonies of 9945A on the same respective media.

DNA was prepared from cells of M28-ST and M28-RT, and the two preparations were tested in transformation experiments with M28. (See experiment 3 in Table 1.) Transformants that were produced with the DNA from the rough transformant were all of the rough colony type, whereas those produced with the DNA from either the smooth transformant or 9945A included both rough and smooth colony types.

Transformation of M28 cells on minimal agar. Although cells of M28 grown for 5 to 16 hr in NBY always gave rise to transformants when spread with DNA on minimal agar plates, usually no transformants were obtained when such cells were incubated with DNA in liquid suspension for 30 min and then spread on minimal agar plates with deoxyribonuclease. This suggested that cells became competent during incubation on minimal agar or that DNA was taken up by the cells over a long period of time. The finding that transformants appeared earlier when cells were grown in NBSG than when they were grown in NBY suggested that those grown in NBSG became competent sooner after being spread on the plates. Therefore, an experiment was done to ascertain the time necessary for such cells to be transformed on agar plates. A series of plates were spread with 0.05 ml (45 μg) of DNA from wild-type 9945A and 0.1 ml (2×10^8 cells) of an 18-hr culture of M28 grown in NBSG. At 1-hr intervals during incubation, representative plates were sprayed with a solution of deoxyribonuclease, and incubation was continued for a total of 72 hr. As evidenced by the absence of colonies, none of the cells was transformed during the first 4 hr. On the plates sprayed at 5 hr, an average of 57 colonies appeared and there was a tenfold increase in the number of transformants during the period between 5 and 11 hr. However, the number of transformants was not maximum even at 11 hr, as evidenced by the larger number (>1,000) of transformants on the control plates that were not sprayed with deoxyribonuclease.

Transformation of M28 cells in liquid suspension. In experiments on transformation of cells in liquid suspension, usually no transformants were obtained with cells grown for periods of time ranging from 5 to 16 hr, although in parallel tests such cultures always produced transformants when samples of the DNA-cell mixtures were plated without deoxyribonuclease. However, if cells which had reached the stationary phase of growth were incubated in minimal broth on a shaker, some of the cells became competent in a few hours, even though viable counts indicated that multiplication of cells did not occur. This suggested that cells became competent sometime after cultures reached the stationary phase of growth, and this led to an experiment in which cells were taken from an NBSG broth culture at 2-hr intervals between 18 and 28 hr and tested for transformation in liquid suspension by the routine procedure described in Materials and Methods. DNA (52 μg) from wild-type 9945A was used. The number of viable cells, 3×10^9 per ml of culture, did not vary significantly in the samples taken at the various times. From 1.5×10^9 recipient cells, 720 transformants were obtained with the 18-hr sample; the number increased to a maximum of 2,200 at 22 hr and decreased gradually to 830 at 28 hr.

More transformants were obtained when the mixtures of 22-hr cells and DNA were shaken than when they were incubated statically (experiment 1; Table 2). Increasing the period of shaking from 30 to 60 min resulted in a further increase in the number of transformants (experiment 2), and the numbers did not change significantly when the time was increased to 2 hr. Washing the cells and resuspending them in minimal broth did not appear to affect the transformation frequency (experiment 2). Table 2 also shows the results obtained with decreasing numbers of recipient cells. As the number of cells decreased, the number of transformants decreased almost proportionately, as one should expect. Under these conditions, 10^{-3} to 2×10^{-3}% of the recipient cells were transformed; this is the highest frequency we were able to obtain consistently for transformation of cells in liquid suspension.

Transformation of doubly marked auxotrophs. Other mutations were induced in M28, and four of the resulting double auxotrophs were tested for transformation of each of the markers (Table 3). Glycine$^+$ transformants were obtained with each of the four mutants, and transformants for three of the other markers, serine, histidine, and

TABLE 2. *Transformation of M28 cells under various conditions**

Expt no.	Recipient cells		9945A DNA (262 μg/ml)	Incubation condition	Time of incubation	Transformants per m
	Amt	No.				
	ml		*ml*		*min*	
1	0.5	1.5×10^9	0.2	Static	30	760
	1.0	3.0×10^9	0.4	Shaken	30	8,290
2	1.0	1.9×10^9	0.4	Shaken	30	9,620
	1.0	1.9×10^9	0.4	Shaken	60	12,800
	1.0	1.9×10^9	0.4	Shaken	120	12,700
	1.0†	1.9×10^9	0.4	Shaken	60	11,100
3	1.0	3.0×10^9	0.4	Shaken	60	>10,000
	1.0	3.0×10^8	0.4	Shaken	60	5,670 (2×10^{-3}%)
	1.0	3.0×10^7	0.4	Shaken	60	570 (2×10^{-3}%)
	1.0	3.0×10^6	0.4	Shaken	60	30 (10^{-3}%)

* Recipient cells were grown for 22 hr in NBSG broth. In experiment 3, the cells were diluted in minimal broth. In control tests, no transformants were produced with DNA that had been incubated with deoxyribonuclease for 15 min. In each experiment, control tests were done in which an equal volume of minimal broth replaced the DNA solution; such samples produced no colonies.

† These cells were collected by centrifugation, washed once with minimal broth, and resuspended in minimal broth before being used in this experiment.

TABLE 3. *Transformation of doubly marked auxotrophs derived from M28**

Recipient cells		9945A DNA (925 μg/ml)	Deoxyribonuclease (2 mg/ml)	Transformants per plate	
Mutant	No. per plate			Glycine marker	Other marker
		ml	*ml*		
M28-D1					
(*gly*⁻-*try*⁻)	1.8×10^8	0.05	0	385	0
	1.8×10^8	0.05	0.05	0	0
M28-D2					
(*gly*⁻-*his*⁻)	3.0×10^8	0.05	0	634	10
	3.0×10^8	0.05	0.05	0	0
M28-D7					
(*gly*⁻-*ser*⁻)	1.8×10^8	0.05	0	60	50
	1.8×10^8	0.05	0.05	0	0
M28-D14					
(*gly*⁻-*leu*⁻)	3.5×10^8	0.05	0	>1,000	>1,000
	3.5×10^8	0.05	0.05	0	0

* The recipient cells were from 16-hr NBSG cultures, and the transformation was done by the plate technique. Transformants for the glycine marker were scored on minimal agar supplemented with the other required amino acid (20 μg/ml), and transformants for the second marker were scored on minimal agar supplemented with glycine (100 μg/ml). The numbers of transformants are average values derived from duplicate plates. Control plates without DNA produced no colonies. Abbreviations: *gly*, glycine; *try*, tryptophan; *his*, histidine; *ser*, serine; *leu*, leucine.

leucine, were also obtained. However, transformants for tryptophan were not detected.

From the above experiment, glycine⁺-serine⁻, glycine⁺-leucine⁻, glycine⁺-histidine⁻, and glycine⁺-tryptophan⁻ transformants were isolated, and 17-hr NBSG broth cultures of these were tested for transformation with wild-type 9945A DNA on minimal agar plates. Again, transform-

ants for serine, leucine, and histidine were obtained, but no transformants for tryptophan were detected.

From the experiments on transformation of the doubly marked mutant, glycine$^-$-leucine$^-$ (rough on minimal agar), transformants were isolated which were leucine$^+$-glycine$^-$ and smooth on minimal agar. Cells of such a transformant could be transformed to glycine independence with DNA from wild-type 9945A or from M28-RT (glycine independent, rough transformant of M28), although the frequencies were lower than those usually obtained with the original M28 (rough on minimal agar). With wild-type DNA, all the transformants detected were smooth; with DNA from the rough transformant, rough as well as smooth transformants were obtained. Spontaneous reversion of cells from rough to smooth was never observed.

DISCUSSION

In these studies, we were able to isolate certain auxotrophic mutants of *B. licheniformis* which were capable of being transformed with DNA from the prototrophic parent, although other auxotrophic mutants derived from the same strain appeared to be nontransformable under the conditions tested. Perhaps a more accurate interpretation of the results is that we found conditions under which these particular mutants were able to achieve competence, but that we did not arrive at the proper conditions for development of competence in the other mutants. This raised the question of whether the transformable mutants became competent as a result of some peculiar physiological characteristic, either related or unrelated to their nutritional requirements, or whether the specific markers in them were particularly amenable to transformation. When each of four other markers, histidine, tryptophan, serine, and leucine, was introduced singly into the transformable glycine auxotroph, three of them, histidine, leucine, and serine, were transformable, whereas the tryptophan marker appeared not to be. This only shows that the transformability was not specific or unique for the glycine marker. The fact that with the double mutant, glycine$^-$-tryptophan$^-$, transformants were detected for glycine but not for tryptophan indicates that this mutant retained the ability to become competent for glycine transformation under the conditions of our tests. It appears that either the tryptophan marker is not transformable or that our conditions did not permit selection of tryptophan$^+$ transformants. Further conclusions must await additional information to be gathered with other mutants and other markers.

Under the best conditions tested only about 10^{-3}% of the recipient cells transformed when cells and DNA were incubated together in liquid suspension. Although the technique of transforming on agar plates appeared to give higher frequencies of transformation (up to 20%; Table 1, experiment 4), these values cannot be taken as actual frequencies. Tests not reported here indicated that several cell divisions occurred after unwashed cells of the glycine mutant were spread on minimal agar plates. Therefore, one cannot be certain that the actual cells put on the plates were the ones that transformed; the frequency values for these experiments indicate the proportion of cells that were competent or gave rise to competent cells.

Takahashi (1962) reported that cells of *B. subtilis* became competent on minimal agar, and under the conditions of his tests cells were maximally competent at 150 min. In our experiments, we did not determine at what time the maximal number of cells were competent. In the experiment in which DNA on the plates was destroyed at various times by spraying with deoxyribonuclease, maximal transformation had not occurred even at 11 hr, which was the longest time tested.

We wish to emphasize that the terms "smooth" and "rough" as used here are descriptive of the colonial phenotypes on minimal agar as a reflection of the accumulation or lack of accumulation, respectively, of glutamyl polypeptide. With the available information, we cannot assess genetically the rough character of the glycine mutant. With respect to glutamyl polypeptide synthesis, perhaps this mutant is either leaky or suppressed, since in the presence of high concentrations of appropriate precursors, as in medium E (Leonard et al., 1958), peptide was synthesized. There is also the possibility that M28 is genotypically smooth and that some other physiological characteristic interferes with peptide synthesis or accumulation on minimal agar. Whatever the nature of the rough character of M28, the results indicate that it is a transformable property, since rough glycine$^+$ transformants were obtained from smooth glycine$^-$ recipient cells. We plan to investigate this aspect of the

problem further; some of the possibilities lend themselves to experimental approach.

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